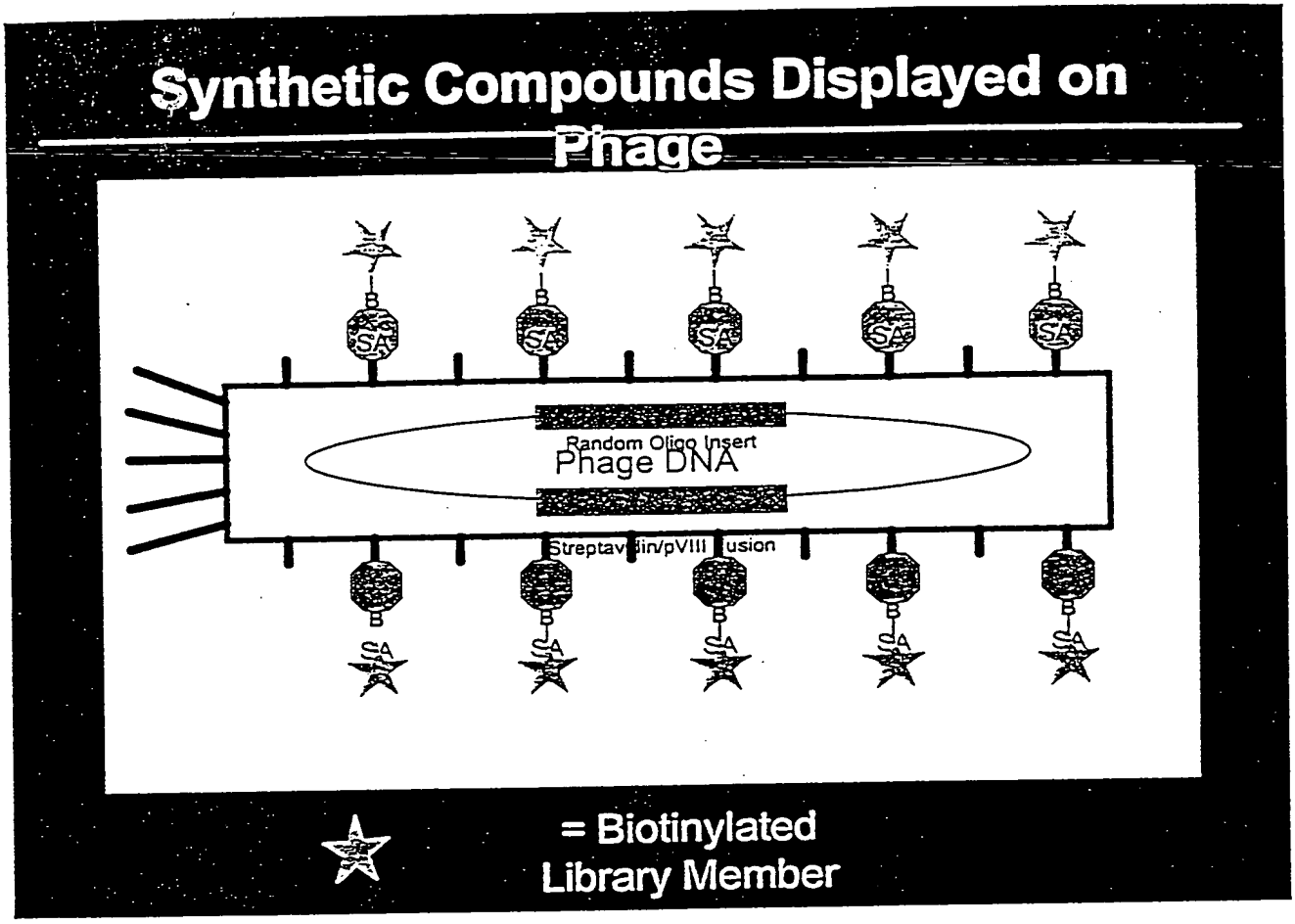
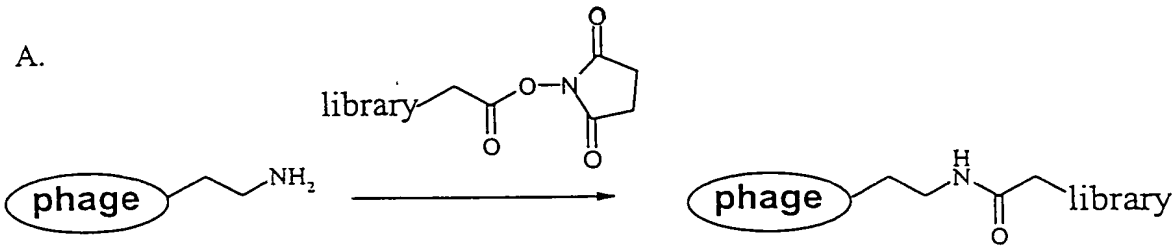
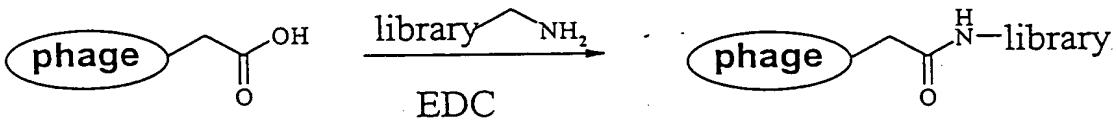

Synthetic Compounds Displayed on Phage
SA
Random Oligo Insert
Phage DNA
Streptavidin/pVIII fusion
= Biotinylated Library Member

FIG. 1

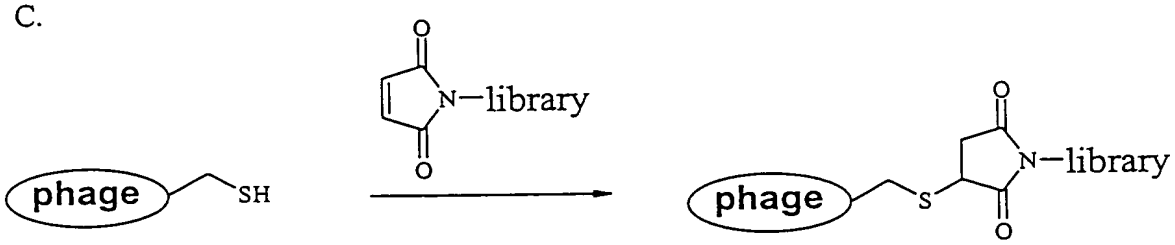
A.


library
O
O
N
O
O
phage
NH2
H
N
library
O

B.


phage
OH
O
library
NH2
EDC
H
N
library
O

C.


O
N
library
O
phage
SH
N
library
S
O

D.

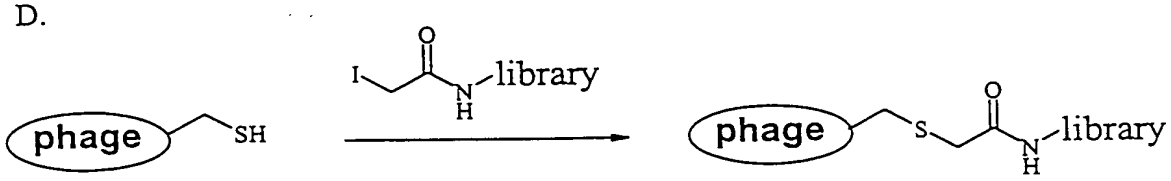
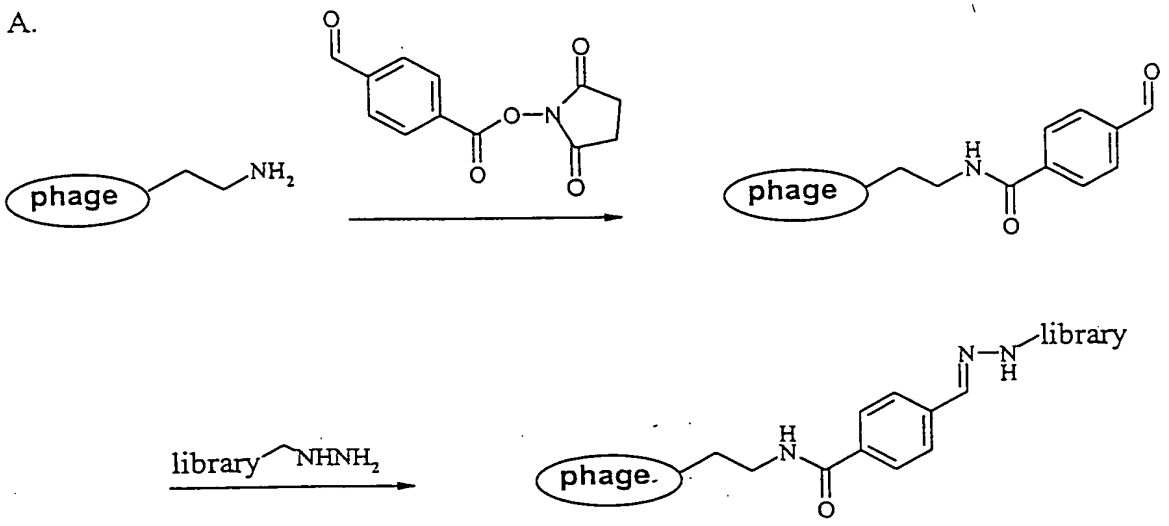

O
I
N
H
library
phage
SH
O
S
N
H
library

FIG. 2


A.
phage
NH2
O
O
O
O
N
O
H
N
O
phage
O
library
N
N
H
H
N
O
phage
library
NHNH2

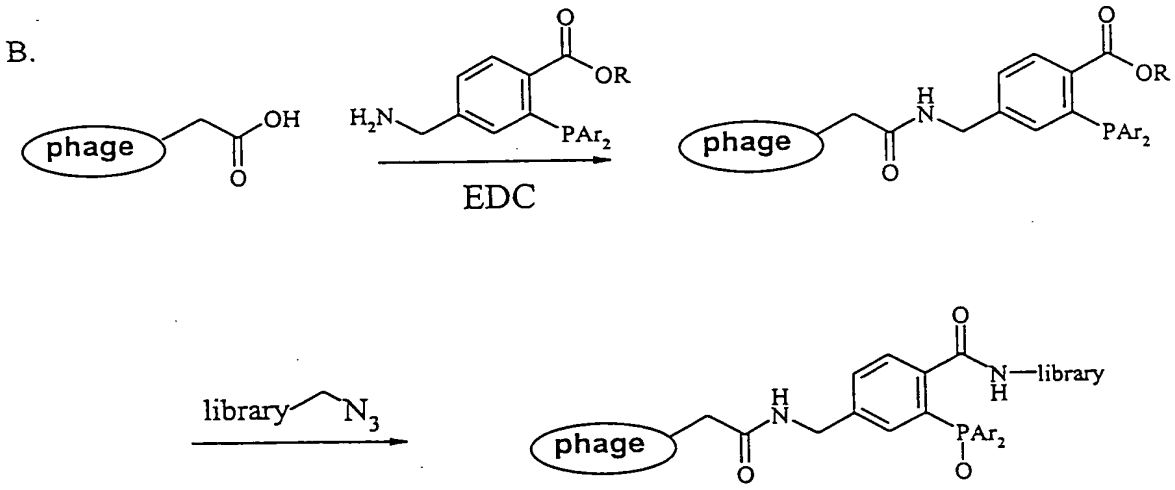

B.
phage
OH
O
H2N
O
OR
PAr2
EDC
phage
H
N
O
O
OR
PAr2
library
N3
phage
H
N
O
O
N
H
library
PAr2
O

FIG. 3

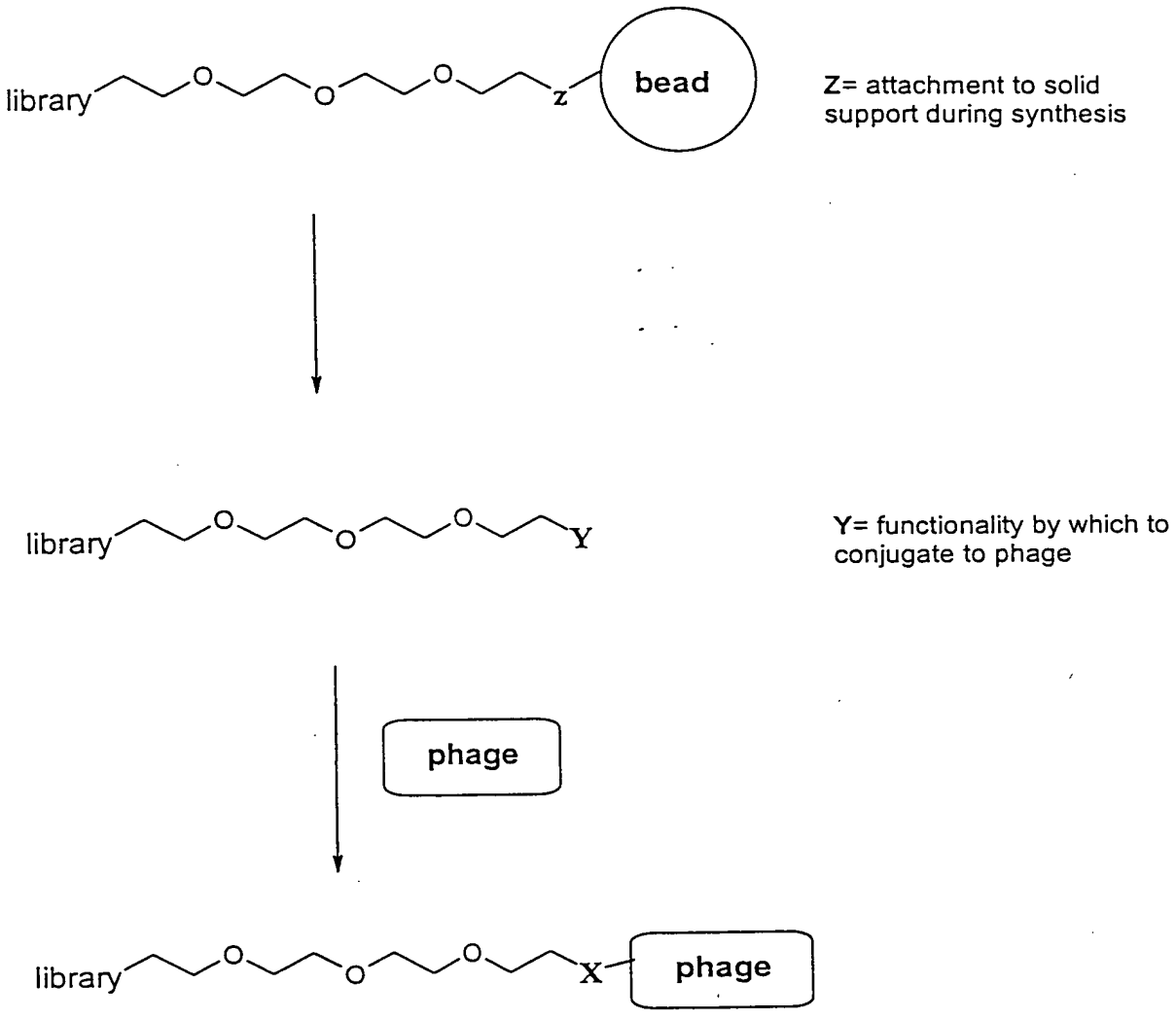

library
O
O
O
z
bead
Z= attachment to solid support during synthesis
library
O
O
O
Y
Y= functionality by which to conjugate to phage
phage
library
O
O
O
X
phage

FIG. 4

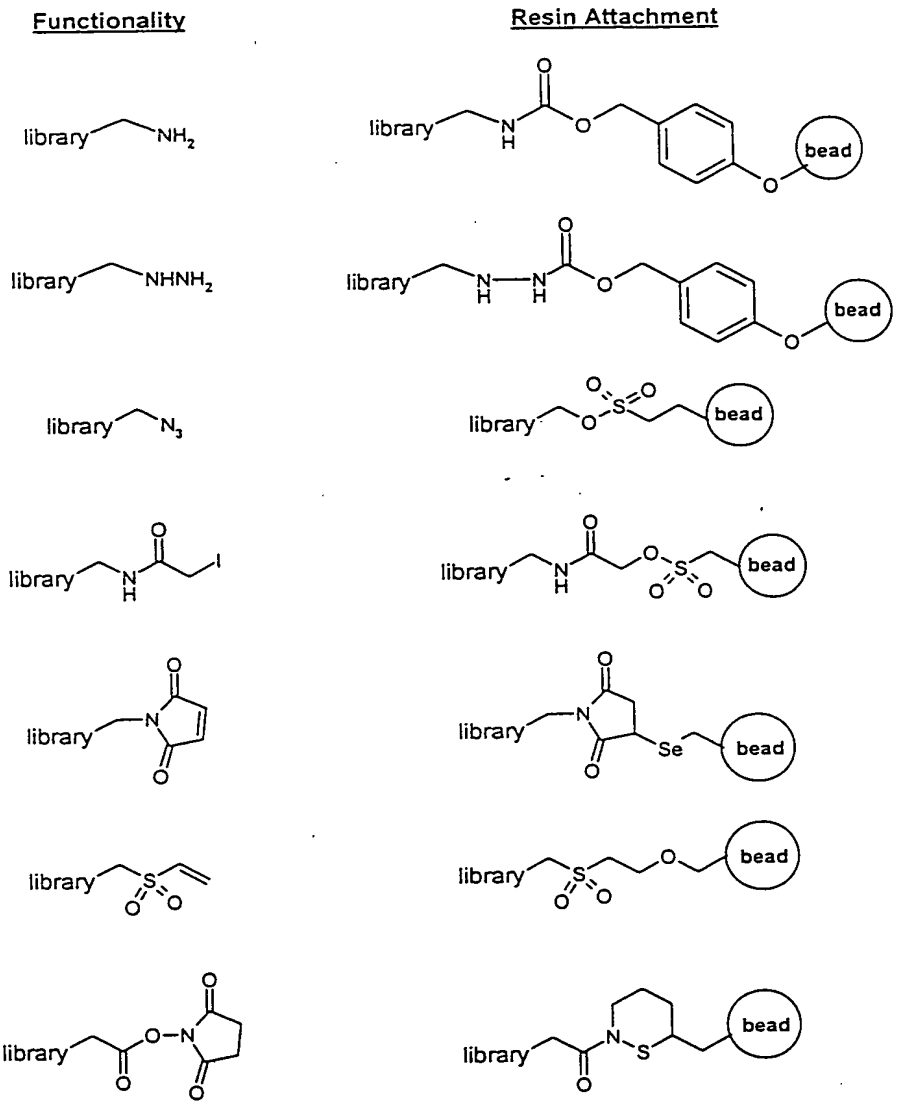

Functionality
Resin Attachment
library
NH2
NHNH2
N3
I
Se
bead

FIG. 5

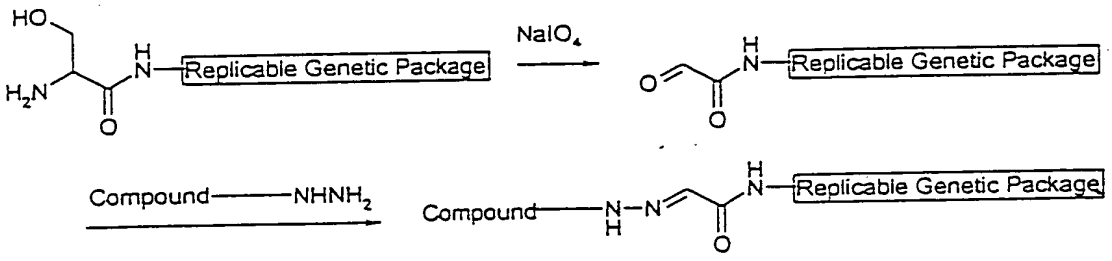

HO
H_2N
H
N
Replicable Genetic Package
O
$NaIO_4$
O
H
N
Replicable Genetic Package
O
Compound——NHNH$_2$
Compound——N
H
N
H
N
Replicable Genetic Package
O

FIG. 6

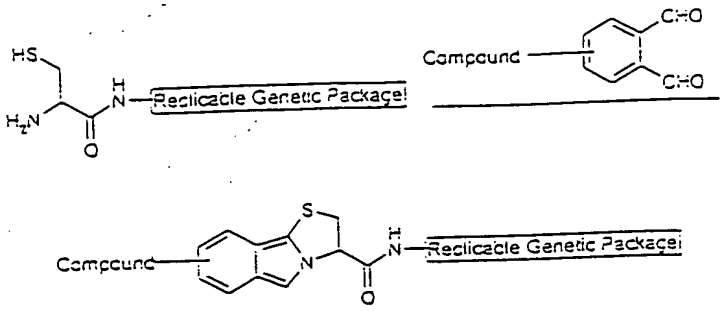


FIG. 7A

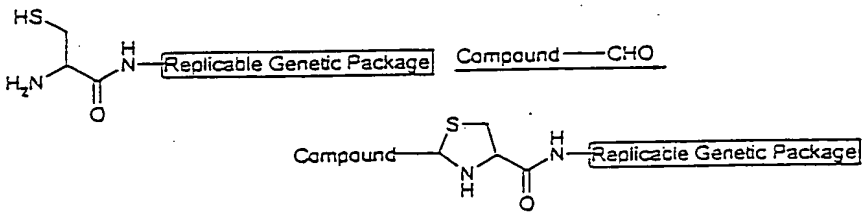


FIG. 7B

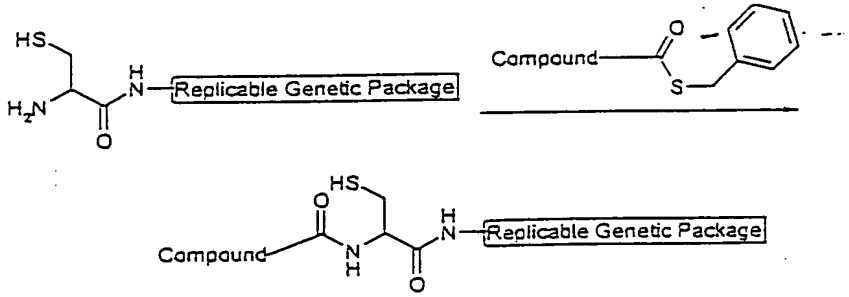


FIG. 7C

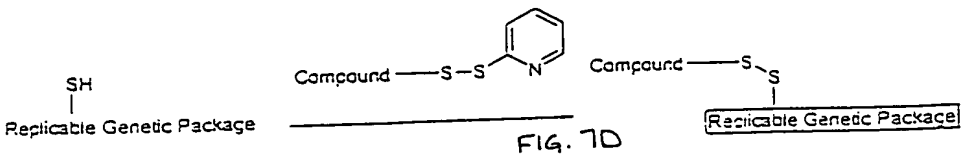


FIG. 7D

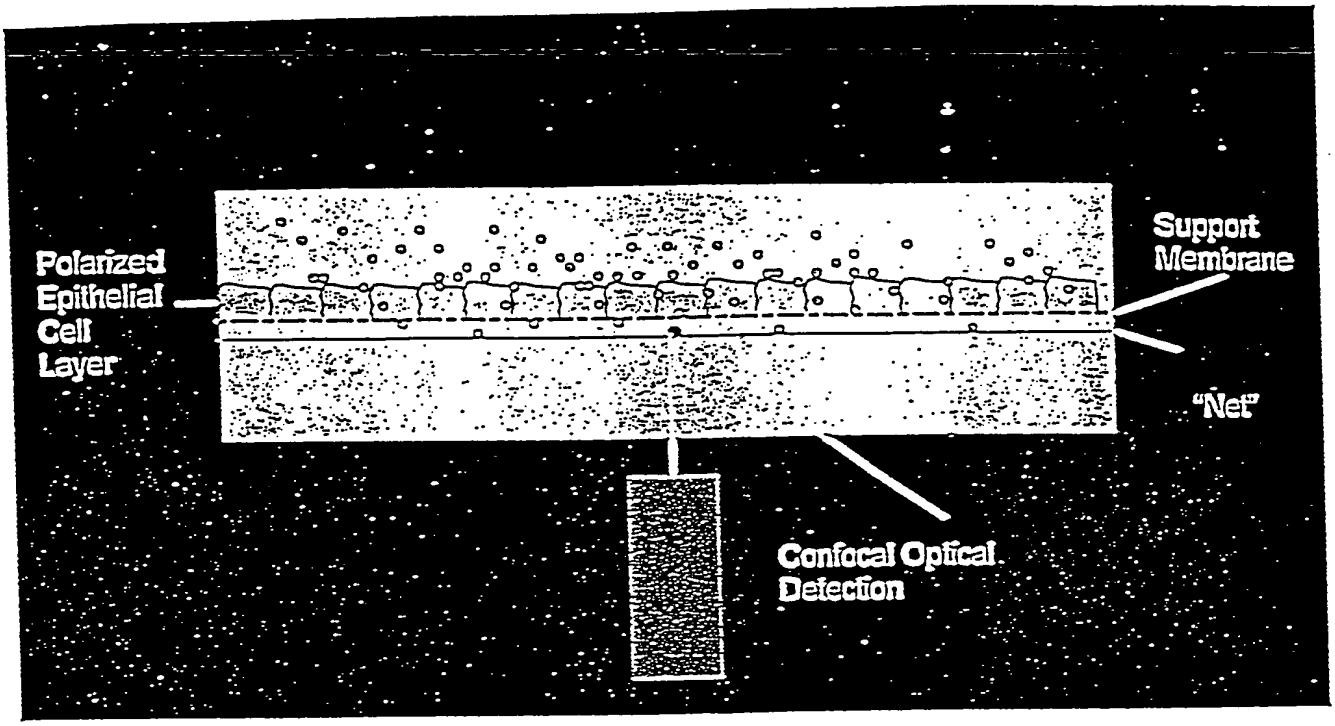

Polarized
Epithelial
Cell
Layer
Support
Membrane
"Net"
Confocal Optical
Detection

FIG. 8

Filamentous phagemid display (gene VIII)

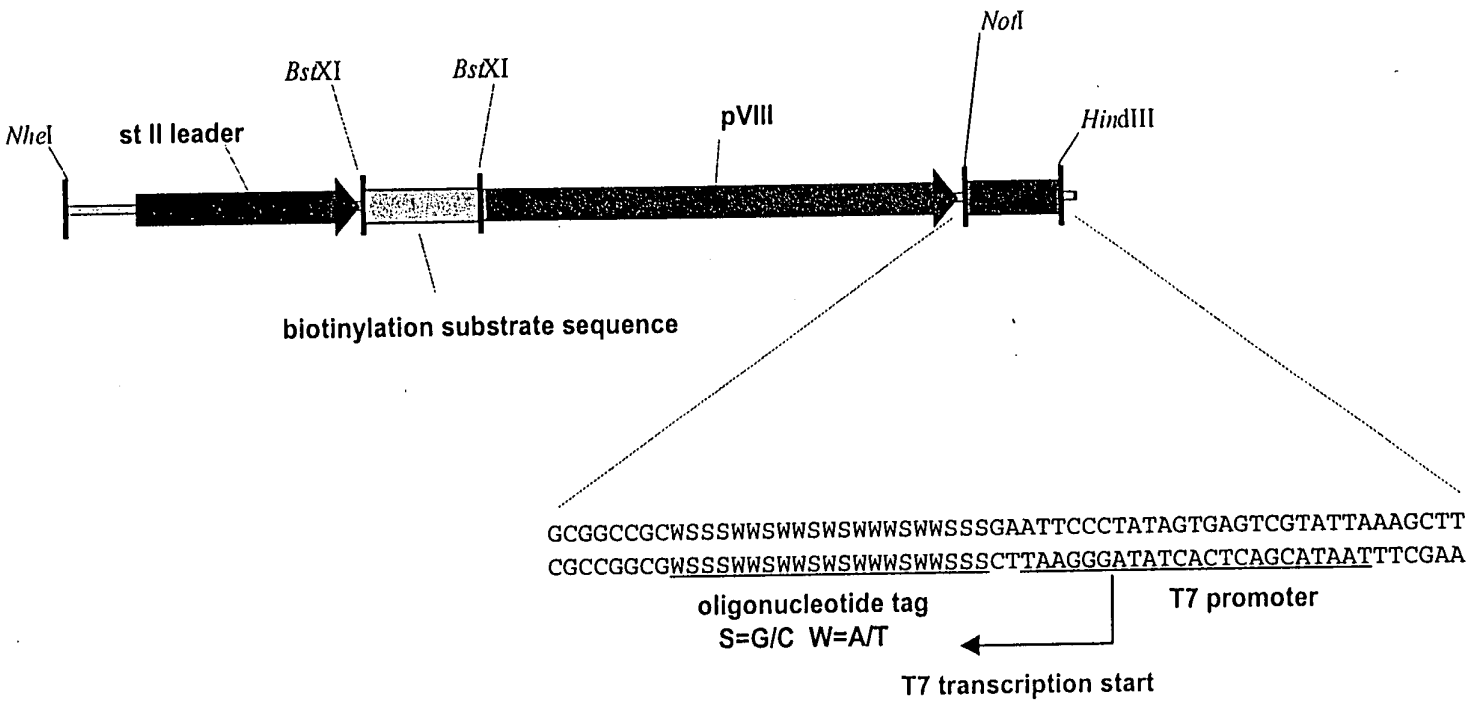


FIG. 9

Filamentous phagemid display (gene VIII)

A.

biotin

G G L N D I F E A Q K I E W H E G G G G S

GGCGGGCTTAATGATATTTTTGAGGCTCAGAAGATTGAGTGGCATGAGGGAGGCGGGGGTAGC . . gene 8

biotinylation substrate sequence flexible spacer

T7 display (gene 10B)

B.

biotin

N S G G G G L N D I F E A Q K I E W H E *

gene 10B . . AATTCTGGAGGCGGGGGTCTTAATGATATTTTTGAGGCTCAGAAGATTGAGTGGCATGAGTAAGTAACTAA

spacer biotinylation substrate sequence

FIG. 10

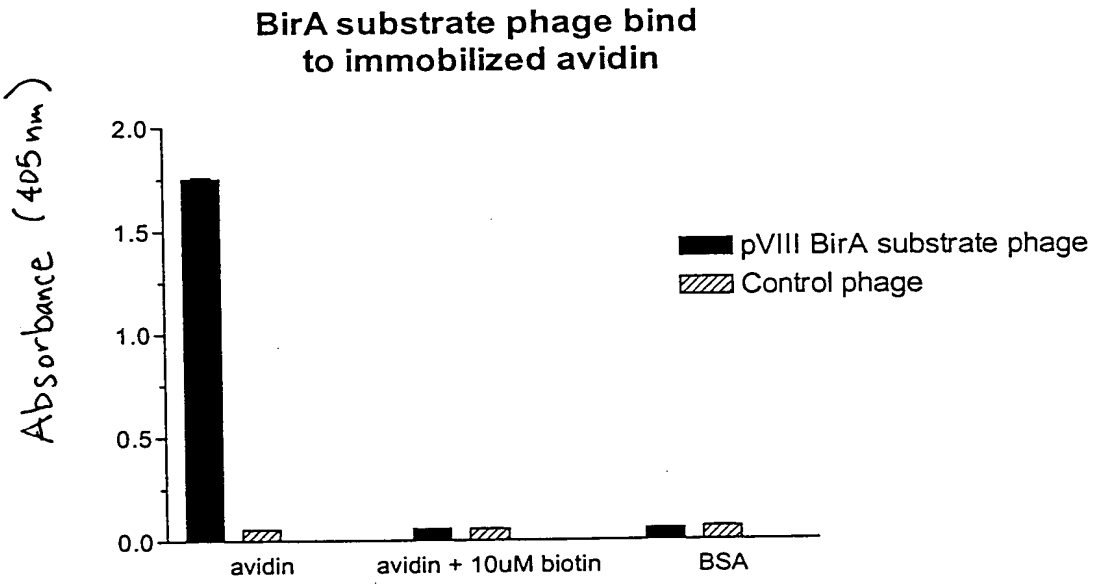

BirA substrate phage bind
to immobilized avidin
Absorbance (405 nm)
2.0
1.5
1.0
0.5
0.0
avidin
avidin + 10uM biotin
BSA
pVIII BirA substrate phage
Control phage

FIG. 11

T7 display (gene 10B)

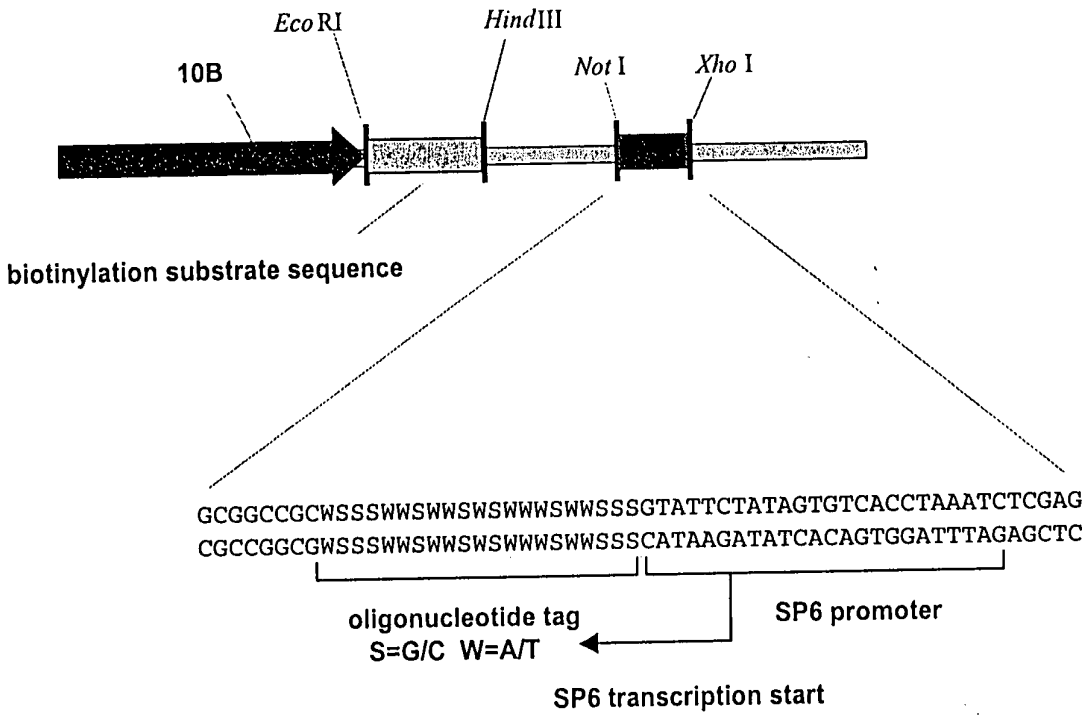


FIG. 12

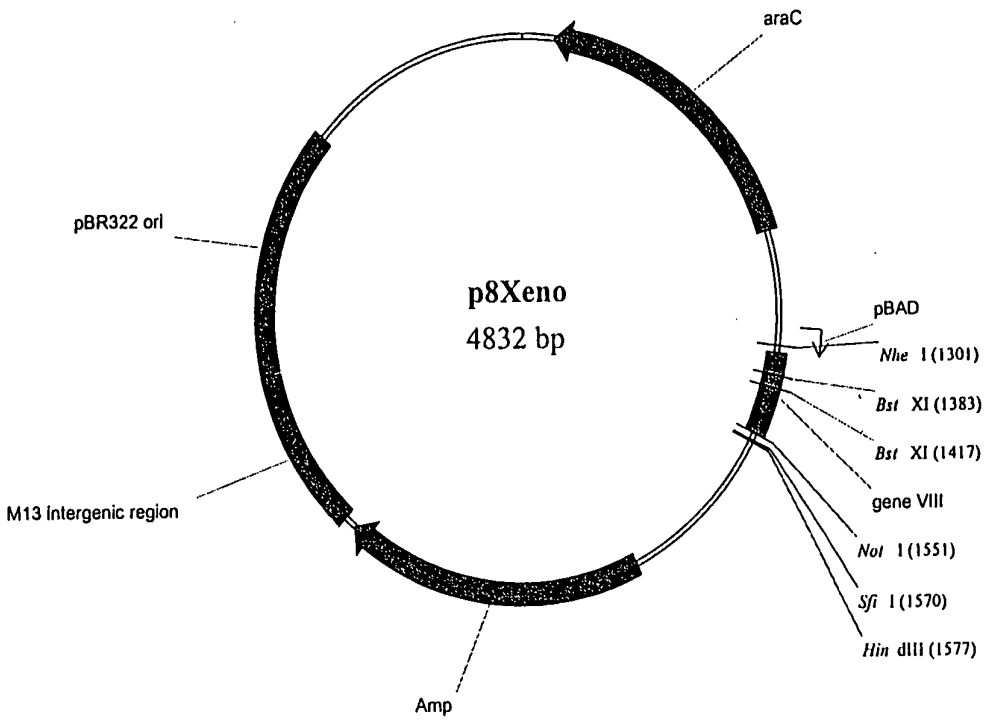

araC
pBR322 ori
p8Xeno
4832 bp
pBAD
Nhe I (1301)
Bst XI (1383)
Bst XI (1417)
gene VIII
Not I (1551)
Sfi I (1570)
Hin dIII (1577)
M13 intergenic region
Amp

FIG. 13

Fig. 14 A

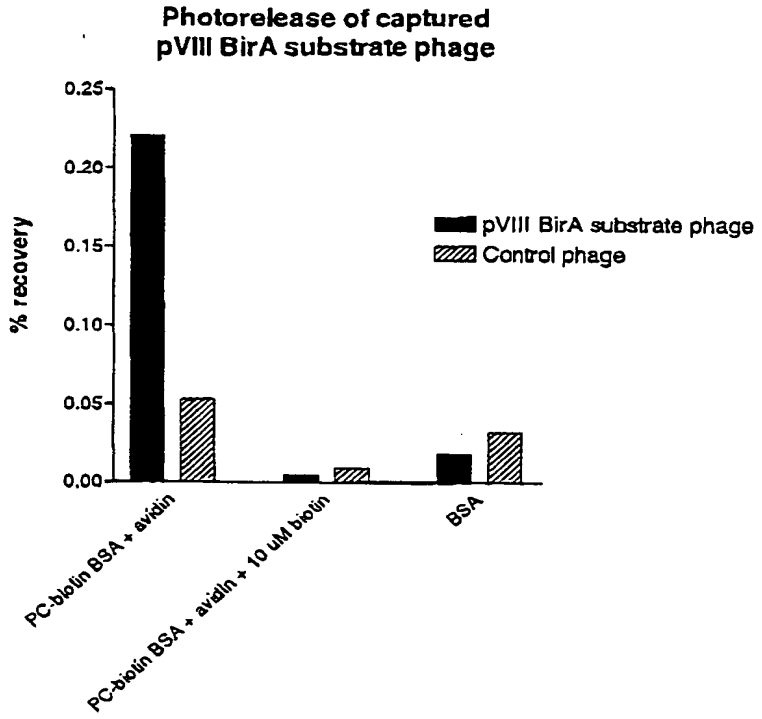
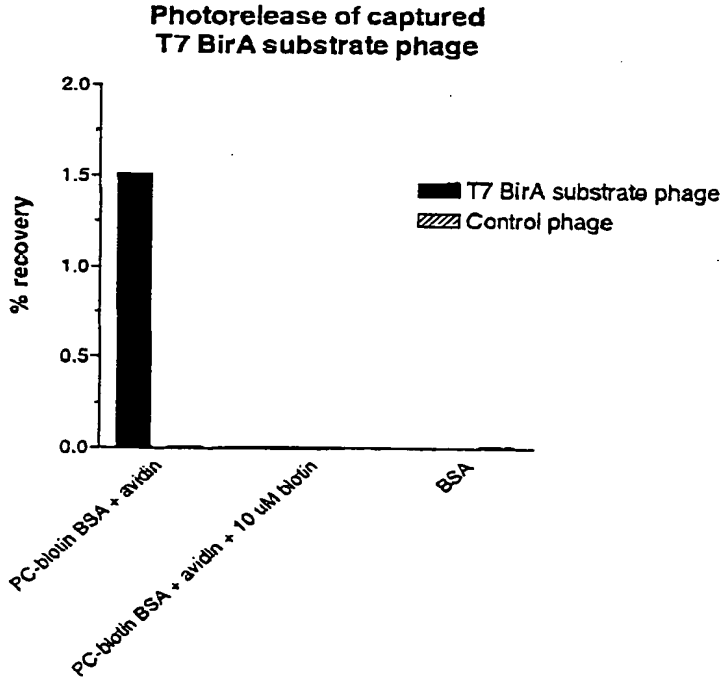


Fig. 14 B



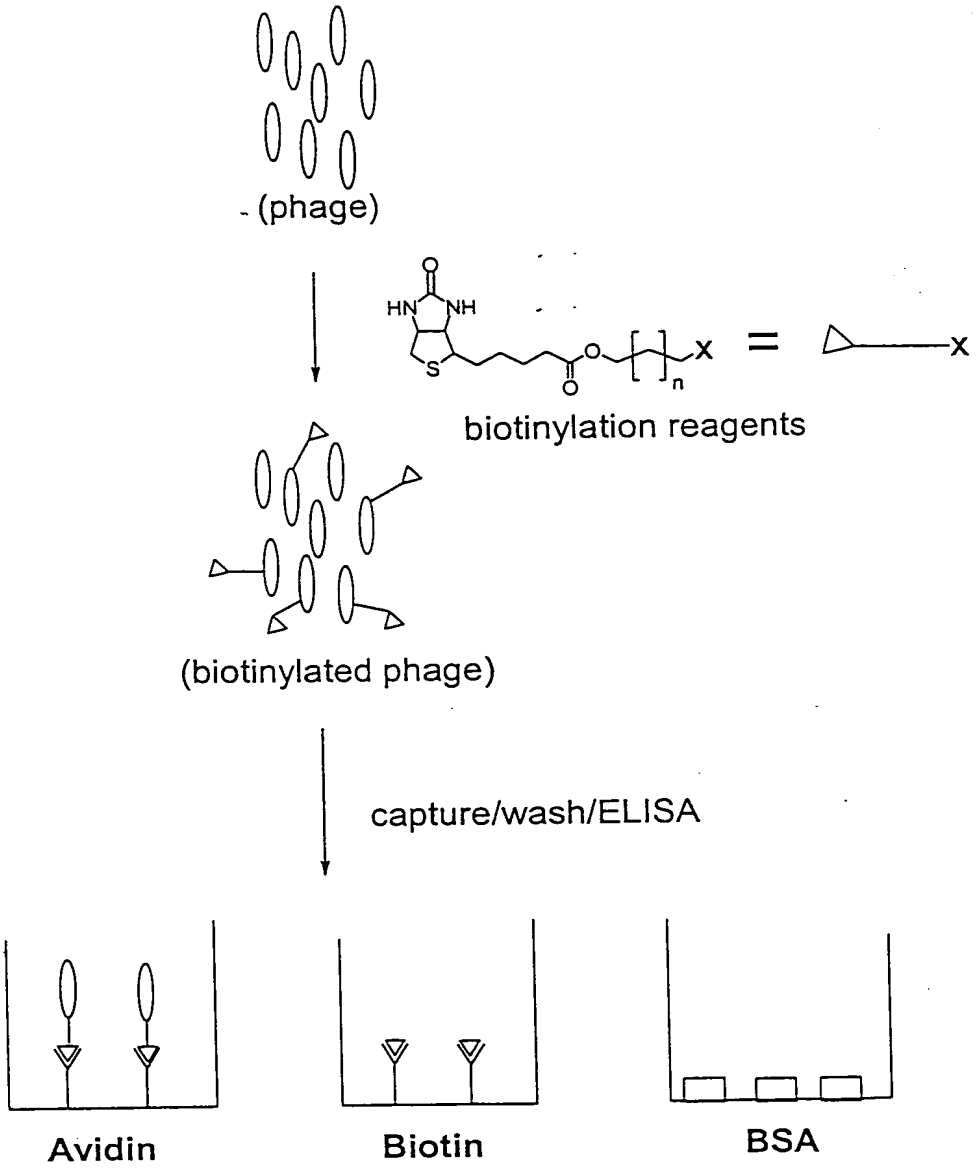
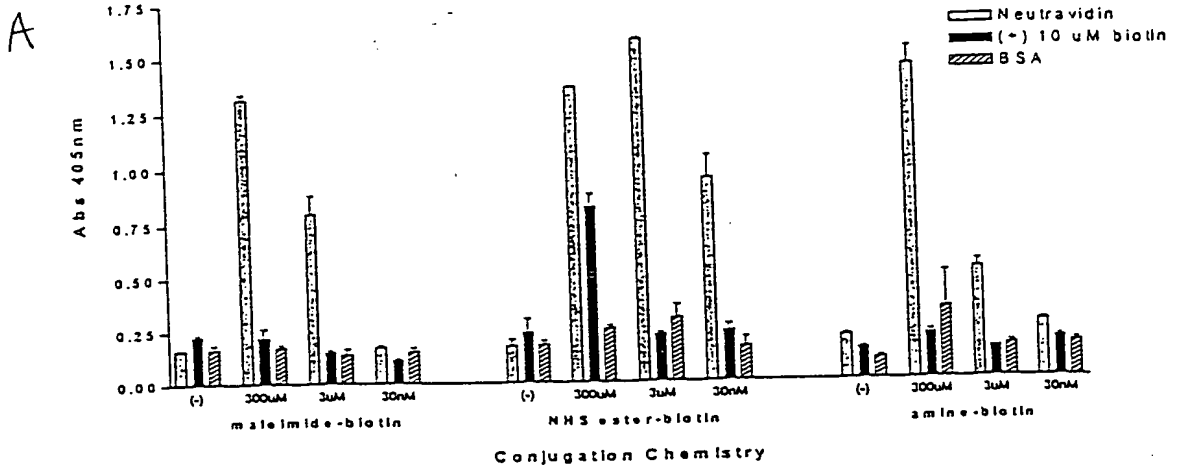
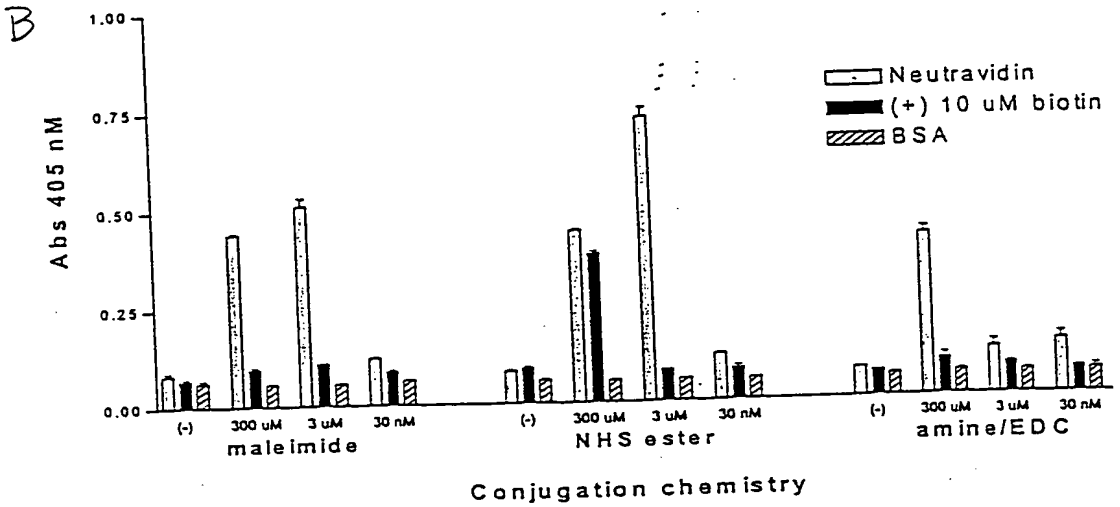

(phage)
HN
NH
S
O
x
n
=
x
biotinylation reagents
(biotinylated phage)
capture/wash/ELISA
Avidin
Biotin
BSA

FIG. 15


A
Abs 405nm
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Neutravidin
(+) 10 uM biotin
BSA
(-)
300uM
3uM
30nM
maleimide-biotin
NHS ester-biotin
amine-biotin
Conjugation Chemistry


B
Abs 405 nM
1.00
0.75
0.50
0.25
0.00
Neutravidin
(+) 10 uM biotin
BSA
(-)
300 uM
3 uM
30 nM
maleimide
NHS ester
amine/EDC
Conjugation chemistry

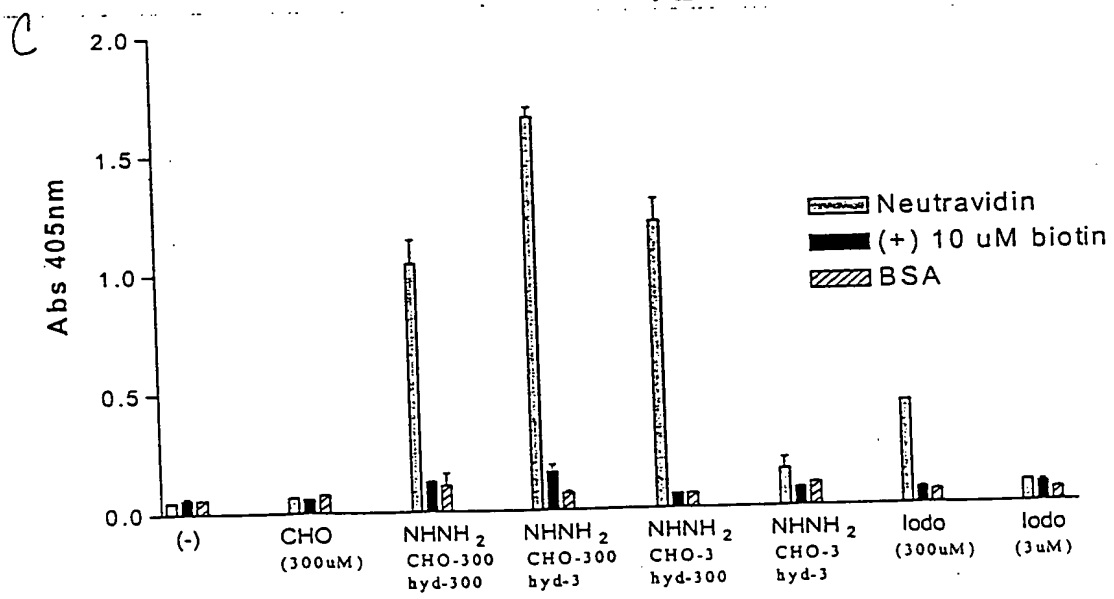

C
Abs 405nm
2.0
1.5
1.0
0.5
0.0
Neutravidin
(+) 10 uM biotin
BSA
(-)
CHO (300uM)
NHNH2 CHO-300 hyd-300
NHNH2 CHO-300 hyd-3
NHNH2 CHO-3 hyd-300
NHNH2 CHO-3 hyd-3
Iodo (300uM)
Iodo (3uM)
Conjugation chemistry

FIG. 16

FIG. 17A

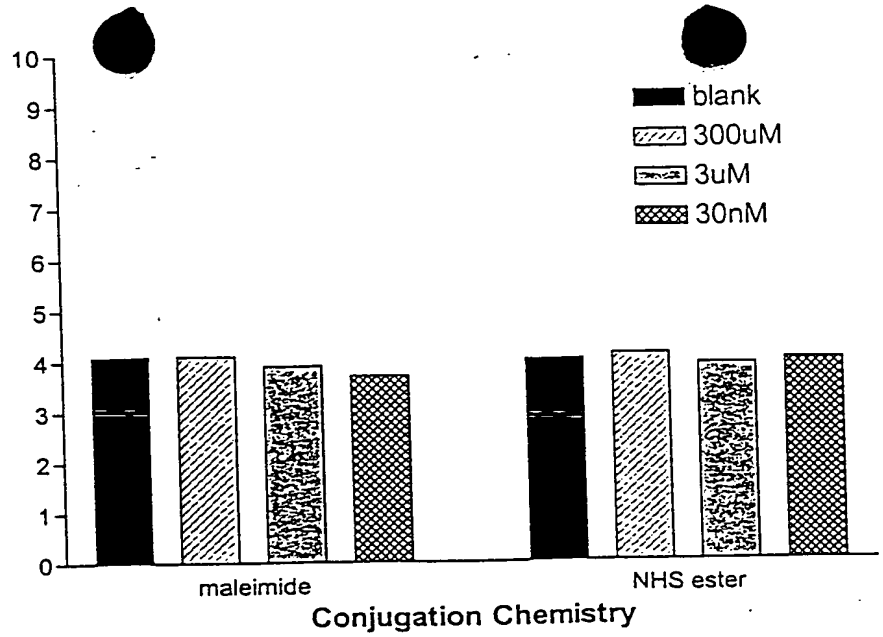


FIG. 17B

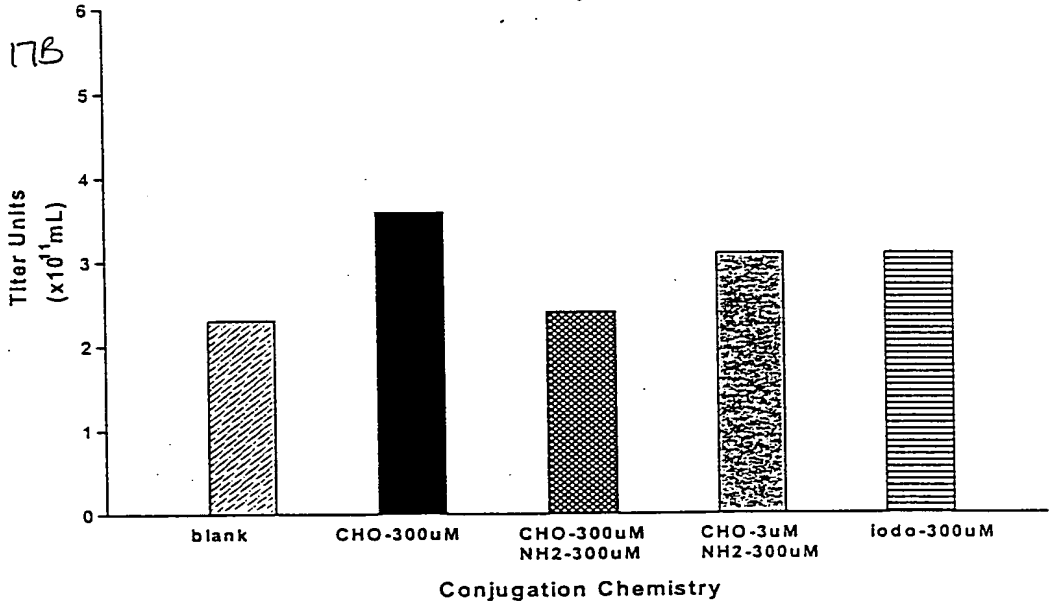


FIG. 17C

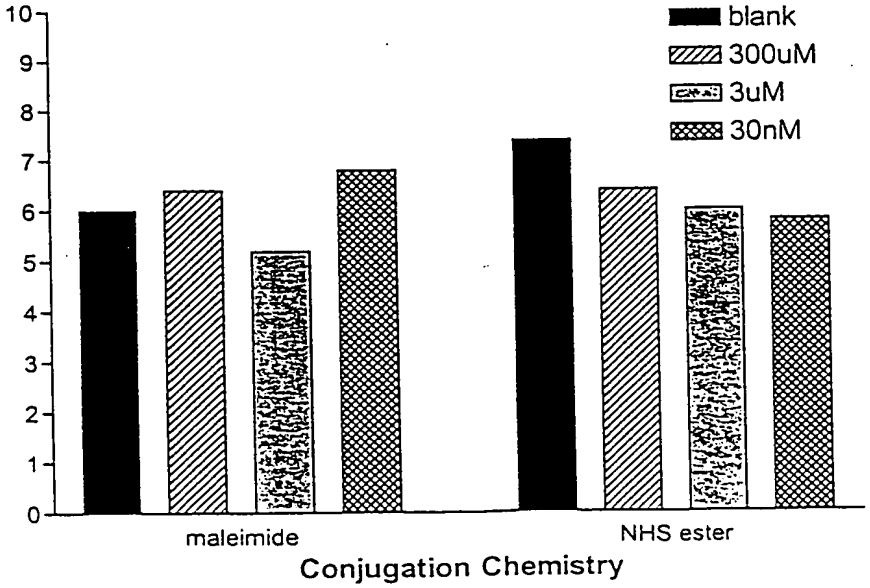
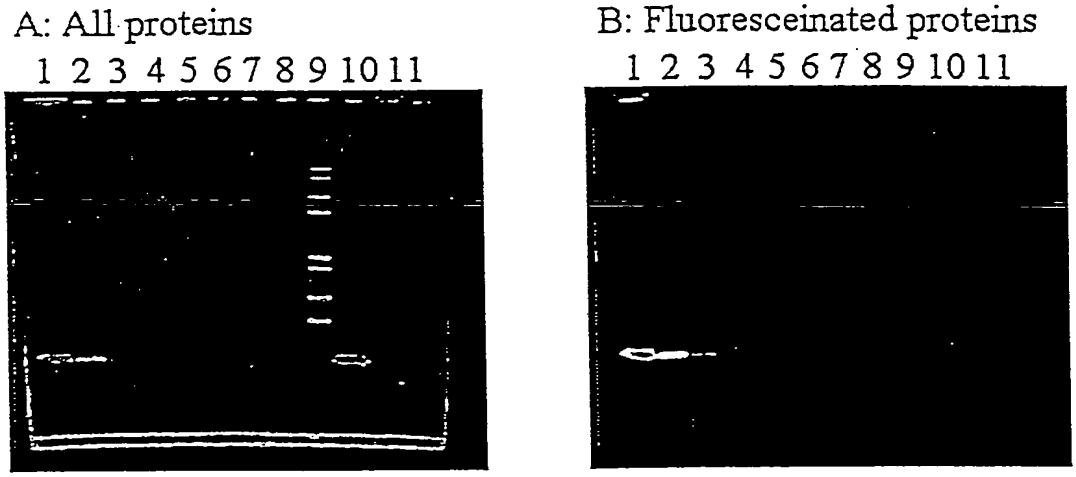
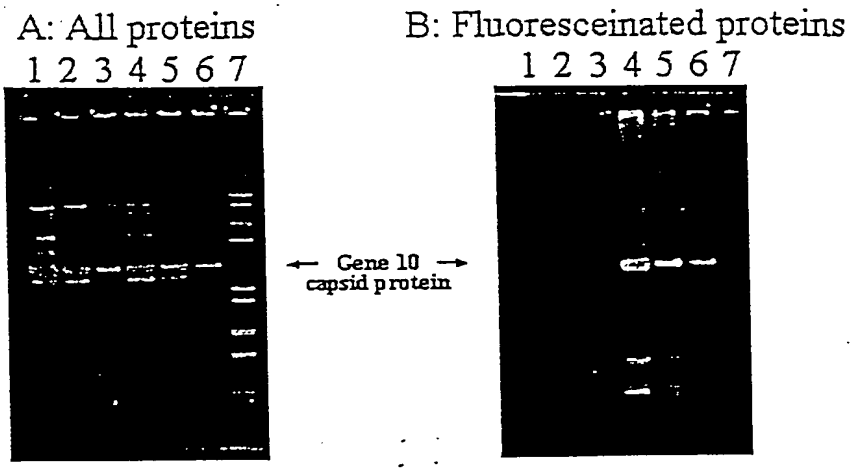


FIG. 18



Lanes 1-8: Fd phage treated with fluorescein-NHS ester (2X dilutions)
Lane 9: MW markers
Lane 10-11: Fd phage treated with fluorescein-CO_2H (10X dilution)

FIG. 19



Lanes 1-3: T7 phage treated with fluorescein-CO_2H (2X dilutions)
Lane 4-6: T7 phage treated with fluorescein-NHS ester (2X dilutions)
Lane 7: MW marker

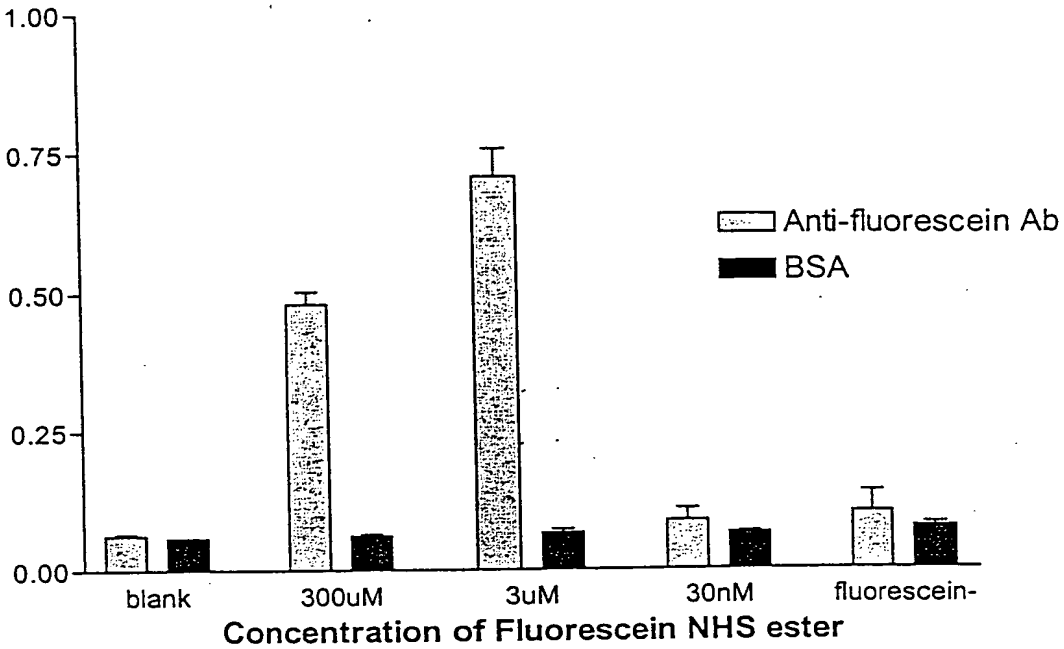

1.00
0.75
0.50
0.25
0.00
Anti-fluorescein Ab
BSA
blank
300uM
3uM
30nM
fluorescein-
Concentration of Fluorescein NHS ester

FIG. 20

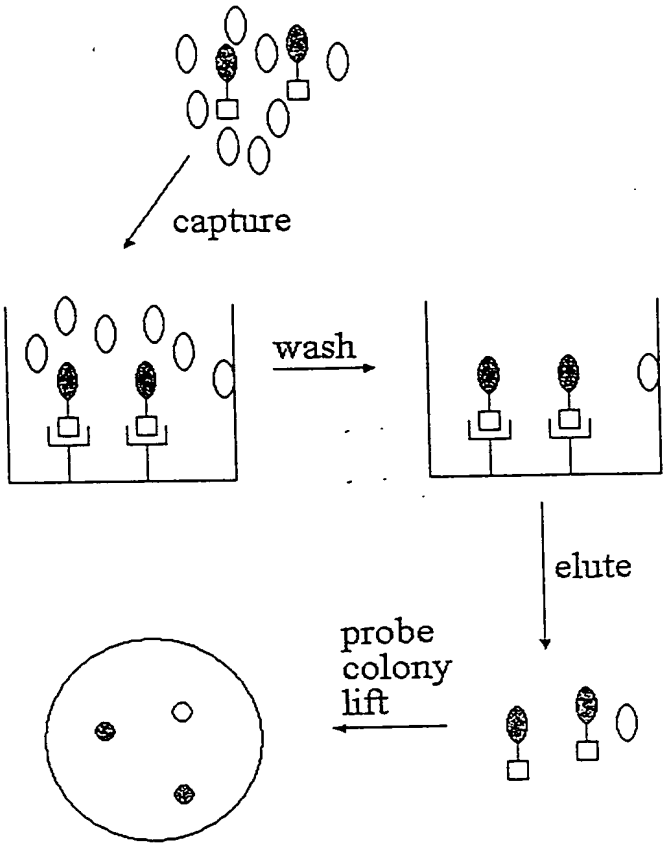


FIG. 21

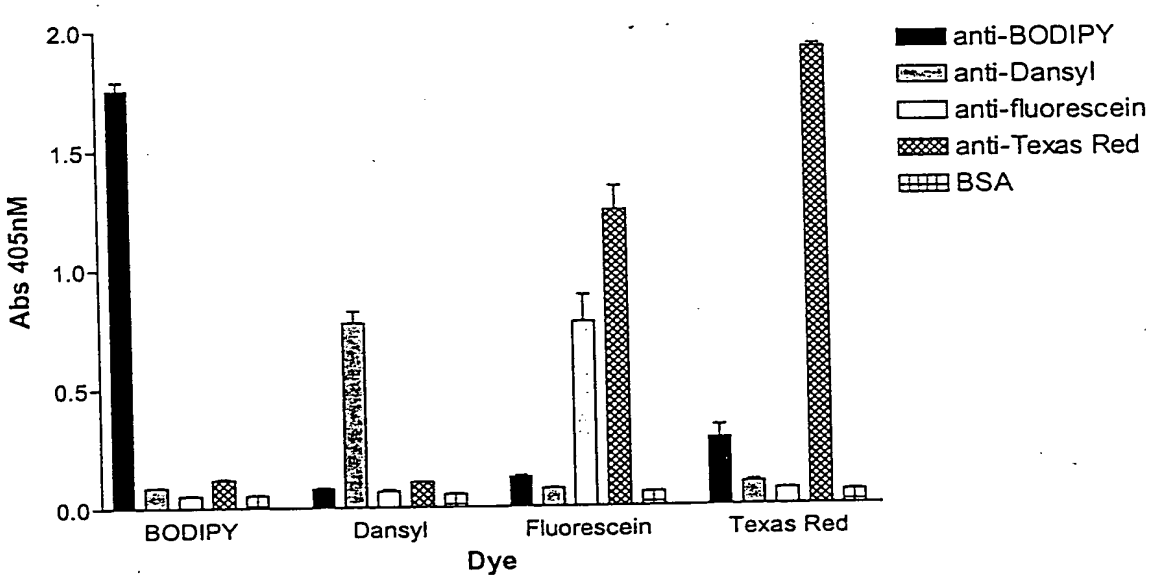

2.0
1.5
1.0
0.5
0.0
Abs 405nM
BODIPY
Dansyl
Fluorescein
Texas Red
Dye
anti-BODIPY
anti-Dansyl
anti-fluorescein
anti-Texas Red
BSA

FIG. 22